

Data Evaluation Report on the Toxicity of Metconazole Technical (Isomer Mix) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number {.....}

EPA MRID Number 468084-24

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	329169
	OECD Data Point	{.....}
	EPA MRID	468084-24
	EPA Guideline	Non-guideline (OECD 204)

Test material: Metconazole (Isomer mix) **Purity:** 97.9% (83.3% *cis* and 14.6% *trans*)
Common name: Metconazole
Chemical name:
 IUPAC: (1*RS*,5*RS*;1*RS*,5*SR*)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol
 CAS: 5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol
 CAS No.: 125116-23-6
 Synonyms: KNF-S-474m; CL 900768; AC 900,768

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation

Signature: *Christie E. Padova*
Date: 3/14/07

Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.

Signature: *Teri S. Myers*
Date: 3/30/07

Primary Reviewer: Sujatha Sankula, Biologist
EPA/OPP/EFED/ERB - I

Date: 4/23/07

Secondary Reviewer(s): Christine Hartless
EPA/OPP/EFED/ERB - I

Date: 6/14/07

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category	{.....}	[For PMRA]
EPA PC Code	{.....}	

Date Evaluation Completed: 6/14/07

CITATION: Mitchell, G.C., *et al.* 1996. Toxicity of AC 900,768 (Metconazole) Technical to Rainbow Trout (*Oncorhynchus mykiss*) in a Flow-Through Prolonged Toxicity Test. Unpublished study performed by T.R. Wilbury Laboratories, Inc., Marblehead, MA. Laboratory Project No. 954-96-129. Study submitted by Valent USA Corporation, Walnut Creek, CA. Study initiated March 1, 1996 and submitted December 10, 1996.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the prolonged toxicity of a pesticide to juvenile fish. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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EXECUTIVE SUMMARY:

The 28-day sub-acute toxicity of metconazole (isomer mix) technical-grade to juvenile rainbow trout (*Oncorhynchus mykiss*) was studied under flow-through conditions. Juvenile rainbow trout (mean total length 55 mm; mean wet weight 1.70 g; 20 fish per level) were exposed to nominal concentrations of 0 (negative and solvent controls), 0.64, 1.2, 2.5, 5.1, and 10 mg/L. Time-weighted average (TWA) concentrations were <0.118 (<LOQ, controls), 0.511, 1.16, 2.53, 5.59, and 8.98 mg ai/L, respectively. The test system was maintained at 15.4-16.0°C and a pH of 7.9 to 8.4. The 28-day LC₅₀ (with 95% C.I.) was 1.71(1.16-2.53) mg ai/L. The 28-day NOAEC was 1.16 mg ai/L, based on mortality and clinical signs of toxicity observed at the ≥2.53 mg ai/L treatment levels.

No mortality or clinical signs of toxicity were observed at the ≤1.16 mg ai/L levels. All surviving fish exhibited signs of toxicity at the 2.53 and 5.59 mg ai/L levels prior to complete mortality by days 8 and 3, respectively. Effects included resting on bottom of test vessel, loss of equilibrium, gasping, change of coloration, erratic swimming, and/or lethargy. Complete mortality occurred at the 8.98 mg ai/L level (highest treatment level) prior to observations of any signs of toxicity. No statistically-significant differences on terminal growth (standard length and wet weight) of surviving fish from the 0.511 and 1.16 mg ai/L levels were observed.

This study does not fulfill any current U.S. EPA guideline requirements. This study, however, is scientifically sound and classified as supplemental, and it provides useful information on the 28-day sub-acute toxicity to rainbow trout (*Oncorhynchus mykiss*).

Results Synopsis

Test Organism Size/Age(mean Weight or Length): Juvenile; mean standard length of 47 mm (range of 42 to 50 mm); mean total length of 55 mm (range of 49 to 59 mm); mean wet weight of 1.70 g (range of 1.15 to 2.16 g)

Test Type (Flow-through, Static, Static Renewal): Flow-through

28-day LC₅₀: 1.71 mg ai/L

95% C.I.: 1.16-2.53 mg ai/L

Probit slope: N/A

95% C.I.: N/A

NOAEC: 1.16 mg ai/L

LOAEC: 2.53 mg ai/L

Endpoint(s) affected: mortality and clinical signs of toxicity

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the OECD Guidelines for the Testing of Chemicals Test No. 204 ("Fish Prolonged Toxicity Test: 14-day Study"). There is no current guideline under OPPTS for a prolonged (≥ 14 day) freshwater fish toxicity study. Notable deviations from standard aquatic laboratory practice and/or OECD Guideline 204 included:

Excessive analytical variation ($>20\%$ among replicates/means of replicates in cases of multiple injections) was observed at the two lowest (i.e., nominal 0.64 and 1.2 mg/L) treatment levels. Variation was 34 and 21%, respectively. Acceptable ranges were obtained for the three highest (i.e., nominal 2.5, 5.1, and 10 mg/L) treatment levels (variation of 12, 6.8, and 16%, respectively).

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material CL 900,768

Description: White powder

Lot No./Batch No. : AC 9339-114

Purity: 97.9% (83.3% *cis* and 14.6% *trans*)

Stability of compound under test conditions: The stability of metconazole in solution under actual use conditions was assessed at all treatment levels by analytical determination of the *cis* isomer at weekly intervals during the study. Analytical variation was excessive (21-34%) at the two lowest treatment levels, indicating instability at these levels. However, at the three highest treatment levels, variation was minimal ($\leq 16\%$; refer to Study Deficiencies section below).

Storage conditions of test chemicals: In the dark at room temperature

Physicochemical properties of Metconazole.

Parameter	Values	Comments
Water solubility at 20EC	15 mg/L	pH not reported
Vapor pressure	13 μ Pa	20°C
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

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2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss*)

Age at test initiation: Juvenile (not further specified)

Size: Mean standard length 47 mm (range 42-50 mm)
Mean total length 55 mm (range 49-59 mm)
Mean wet weight 1.70 g (range 1.15-2.16 g)

Source: Mount Lassen Trout Farm, Red Bluff, CA

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 7-day static-renewal range-finding test was conducted at nominal test levels of 0 (negative and solvent controls), 0.0010, 0.010, 0.10, 1.0, and 10 mg/L. Cumulative survival was 100% at the ≤ 1.0 mg/L levels and 0% at the 10 mg/L level.

b. Definitive study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	3 weeks	During the 14-day period before the start of the test, the temperature range was 14.2-14.9°C, and the dissolved oxygen concentration was ≥ 10.0 mg/L.
Conditions (same as test or not):	Same as test	
Feeding:	Commercial flake food (Tetra Min®, lot no. TM06) daily, except for the 48 hours immediately preceding test initiation.	
Health (any mortality observed):	Fish were free of apparent disease, injuries, and abnormalities. Mortality was 0% during the 48 hours prior to test initiation.	
Number of organisms in each treatment at test initiation	20 fish per level, divided equally into two replicate vessels	OECD requires a minimum of 10 fish per level.
Biomass loading rate	1.53 g/L (Instantaneous) and 0.21 g/L/24 hours based on the average flow rate.	

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Parameter	Details	Remarks
		Criteria
<u>Concentration of test material</u> nominal: measured: TWA:	0 (negative and solvent controls), 0.64, 1.2, 2.5, 5.1, and 10 mg/L <0.118 (<LOQ, controls), 0.500, 1.14, 2.52, 5.59, and 8.98 mg ai/L <0.118 (<LOQ, controls), 0.511, 1.16, 2.53, 5.59, and 8.98 mg ai/L	Water samples were collected for analysis on study days 0, 7, 14, 21, and 28; when results were outside the acceptable range (70-120% of day 0 nominal), then the samples were re-extracted and analyzed (up to three total analyses). Results of all analyses were used in the calculation of the mean concentrations. Mean-measured concentrations were 79, 97, 102, 111, and 91% of nominal levels, respectively. Time-weighted average (TWA) concentrations were calculated by the reviewer (using Excel software) and are provided in Appendix II below.
Solvent (type, percentage, if used)	Dimethylformamide 0.1 mg/L	
<u>Number of replicates</u> control: solvent control: treated ones:	2 2 2/level	Four replicate vessels are typically recommended in aquatic fish studies; however, this study design fulfills OECD requirements.
<u>Test condition</u> static renewal/flow-through: type of dilution system for flow through method: flow rate: renewal rate for static renewal:	Flow-through Proportional diluter 7.3 volume additions/24 hours N/A	The diluter was calibrated before and after the test, and monitored twice per day during testing for normal operation.
Aeration, if any	Not required during testing.	

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Parameter	Details	Remarks
		Criteria
Duration of the test	28 days	
<u>Test vessel</u> type/material: (glass/stainless steel) size: fill volume:	Glass 20 L 15 L (18-cm depth)	
Source of dilution water	Carbon-filtered deionized water adjusted to a hardness of 160-180 mg/L as CaCO ₃ . The dilution water was stored in 500-gal polyethylene tanks where it was aerated and continuously passed through a particle filter, UV sterilizer, and activated carbon.	Results from the characterization of a representative sample of deionized water (analyzed approx. 7 months prior to the definitive study) used to prepare the laboratory dilution water was provided. No chemical contaminants were detected.

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Parameter	Details	Remarks
<div>Criteria</div>		
<u>Water parameters</u>		
hardness:	164-168 mg/L as CaCO ₃	
pH:	7.9-8.4	
dissolved oxygen:	7.2-10.0 mg/L	
temperature (s):	15.4-16.0°C	
photoperiod:	16 hours light:8 hours dark, with 15-minute transition periods	
other measurements:	Conductivity: 540-620 μmhos/cm Alkalinity: 133-138 mg/L as CaCO ₃	
interval of water quality measurements:	DO, pH, temperature, and conductivity were measured daily in each vessel that contained live animals. Temperature was also continuously monitored in one test vessel. Hardness and alkalinity were measured at the beginning and end of the study in one control vessel.	
<u>Feeding</u>		Fish were only fed once on day 28.
type/source of feed:	Commercial flake food (Tetra Min®, Lot No. TM06)	
amount given:	A sufficient quantity to be ingested immediately	
frequency of feeding:	Three times daily	

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Parameter	Details	Remarks
		Criteria
Stability of chemical in the test system	Results from water samples collected weekly during the study indicated that the stability of metconazole in solution generally increased with increasing concentration, although due to total mortality at the three highest treatment levels, test sampling did not continue past 14 days. Analytical variation was 34, 21, 14, 7, and 16% for the nominal 0.64, 1.2, 2.5, 5.1, and 10 mg/L levels, respectively (reviewer-calculated).	Variation >20% among replicate means is generally unacceptable.
Recovery of chemical:	105% of nominal	
Frequency of measurement:	Days -1, 0, 7, 14, 21, and 28	
LOD:	Not reported	
LOQ:	<0.118 mg ai/L	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks
		Criteria
Parameters measured including the sublethal effects/toxicity symptoms	<ul style="list-style-type: none"> - Mortality - Sub-lethal effects - Standard length of surviving fish - Wet weight of surviving fish 	
Observation intervals:	Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	N/A	

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II. RESULTS AND DISCUSSION

A. MORTALITY:

Cumulative mortality was 0% in the negative control, solvent control, 0.500, and 1.14 mg ai/L test levels (mean-measured), and 100% in the 2.5, 5.59, and 8.98 mg ai/L test levels. Complete mortality occurred at the highest treatment level (i.e., 8.98 mg ai/L) by day 1. The 28-day LC_{50} (with 95% C.I.) based on the mean measured concentrations was 1.69 (1.14-2.52) mg ai/L. The 28-day Threshold Level of Lethal Effect (TLLE) was reported to be 2.52 mg ai/L.

Table 3: Effect of Metconazole Technical (Isomer Mix) on Mortality of Rainbow Trout.

Treatment (mg ai/L) Mean-measured (and nominal) conc.	No. of fish at start of study	Observation period							
		Day 2		Day 7		Day 14		Day 28	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Control (dilution water only)	20	0	0	0	0	0	0	0	0
Solvent control	20	0	0	0	0	0	0	0	0
0.500 (0.64)	20	0	0	0	0	0	0	0	0
1.14 (1.2)	20	0	0	0	0	0	0	0	0
2.52 (2.5)	20	1	5	19	95	20	100	---	---
5.59 (5.1)	20	13	65	20	100	---	---	---	---
8.98 (10)	20	20	100	---	---	---	---	---	---
NOAEC		1.14							
LC_{50} (95% C.I.)		1.69 (1.14-2.52)							
Positive control, if used mortality: LC_{50} :		N/A							

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B. SUB-LETHAL TOXICITY AND OTHER EFFECTS:

No sub-lethal signs of toxicity were observed at the ≤ 1.14 mg ai/L test levels. All surviving fish exhibited signs of toxicity at the 2.52 and 5.59 mg ai/L levels prior to complete mortality by days 8 and 3, respectively. Effects included resting on bottom of test vessel, loss of equilibrium, gasping, change of coloration, erratic swimming, and/or lethargy. Complete mortality occurred at the 8.98 mg ai/L level (highest treatment level) prior to observations of any signs of toxicity. No statistically-significant differences on terminal growth (standard length and wet weight) were observed between pooled controls and surviving fish from the 0.500 and 1.14 mg ai/L treatment levels. The 28-day EC_{50} (with 95% C.I.) was 1.69 (1.14-2.52) mg ai/L. The 28-day Threshold Level of Observed Effect (TLOE) was reported as 2.52 mg ai/L (refer to Reviewer's Comments section).

Table 4: Effect of Metconazole Technical (Isomer Mix) on Growth of Juvenile Fish

Treatment (mg ai/L) Mean-measured (and nominal) conc.	Growth – standard length (cm)	Growth – weight weight (g)
Control (dilution water only)	51.8	2.29
Solvent control	50.1	2.12
0.500 (0.64)	51.9	2.19
1.14 (1.2)	51.1	2.09
2.52 (2.5)	---	---
5.59 (5.1)	---	---
8.98 (10)	---	---
NOAEC	1.14	1.14
LOAEC	>1.14	>1.14
EC_{50}	>1.14	>1.14
Positive control, if used	N/A	N/A
mortality: EC_{50} : NOAEC		

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C. REPORTED STATISTICS:

The 28-day LC₅₀ and EC₅₀ values with associated 95% confidence intervals were calculated by the binomial/non-linear interpolation method using mean-measured concentrations. The EC₅₀ values were calculated using the number of live, unaffected organisms.

The endpoints of survival at 28 days, and standard length and wet weight of surviving fish were analyzed using TOXSTAT 3.3 (1990) and mean-measured concentrations. Survival data were analyzed using Fisher's Exact Test. Control and vehicle control data for standard length and wet weight were compared with a parametric t-test and in neither case were difference observed; therefore, the controls were pooled for subsequent analyses. A Chi-square test was then used to determine if the data were normally distributed. If the data were normally distributed (wet weights), then a parametric one-way analysis of variance (ANOVA) and Bonferroni's t-test were used to compare the treatment and pooled control data. If the data were not normally distributed (standard lengths), then a non-parametric analysis of variance (Kruskal-Wallis test) was used. The Threshold Level of Lethal Effects (TLLE), Threshold Level of Observed Effect (TLOE), and No Observed Effect Concentration (NOEC) were assigned based on significance (see Reviewer's Comments section).

28-day LC ₅₀ : 1.69 mg ai/L	95% C.I.: 1.14-2.52 mg ai/L
28-day EC ₅₀ : 1.69 mg ai/L	95% C.I.: 1.14-2.52 mg ai/L

TLLE = 2.52 mg ai/L
TLOE = 2.52 mg ai/L
NOEC = 1.14 mg ai/L

D. VERIFICATION OF STATISTICAL RESULTS:

The reviewer verified the 28-day LC₅₀ using the binomial probability method via Toxanal statistical software; the NOAEC based on mortality could be verified visually, as the top three doses produced 100% mortality and the bottom two (and controls) produced none. Additionally, the reviewer verified that there were no significant effects on length or wet weight of surviving fish. Prior to determining the NOAEC, the solvent control and negative control groups were compared using a Student's t-test. No differences were detected for either endpoint, so the negative control data were used for comparison to treatment. These data were analyzed first using the Chi-square and Shapiro-Wilks tests for normality and Hartley and Bartlett's tests for homogeneity of variances. All data satisfied the assumptions of ANOVA, so the NOAEC and LOAEC values were determined using this test, followed by Dunnett's test via Toxstat statistical software. The reviewer based all toxicity calculations on the time-weighted average concentrations; these results are reported in the Executive Summary and Conclusions sections.

28-day LC ₅₀ : 1.71 mg ai/L	95% C.I.: 1.16-2.53 mg ai/L
Probit slope: N/A	95% C.I.: N/A
NOAEC: 1.16 mg ai/L	
LOAEC: 2.53 mg ai/L	
Endpoint(s) affected: mortality and clinical signs of toxicity	

E. STUDY DEFICIENCIES:

This study was not designed to fulfill any current U.S. EPA guideline. The only notable deviation from standard aquatic laboratory practice and/or OECD Guideline 204 was excessive analytical variation (>20% among replicates/means of replicates in cases of multiple injections) observed at the two lowest (i.e., nominal 0.64 and 1.2 mg/L) treatment levels. Variation at these levels was 34 and 21%, respectively. Acceptable ranges were obtained for the three highest (i.e., nominal 2.5, 5.1, and 10 mg/L) treatment levels (variation of 12, 6.8, and 16%,

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respectively). As variation was just slightly above 20% at the NOAEC level of 1.2 mg/L (nominal), and was only 14% at the LOAEC level of 2.5 mg/L, this deviation is not considered to have a significant effect on the results of the study. This study is therefore considered scientifically sound and provides supplemental information on the 28-day sub-acute toxicity of metconazole to rainbow trout.

F. REVIEWER=S COMMENTS:

The reviewer's conclusions were similar to the study author's; however, the reviewer's results are based on the TWA concentrations, rather than the arithmetic mean-measured concentrations. The reviewer reported results are presented in the Executive Summary and Conclusions sections.

The time-weighted averaged were determined using the following equation:

$$\frac{C_0 \left(\frac{T_1 - T_0}{2} \right) + C_1 \left[\left(\frac{T_1 - T_0}{2} \right) + \left(\frac{T_2 - T_1}{2} \right) \right] + C_2 \left[\left(\frac{T_2 - T_1}{2} \right) + \left(\frac{T_3 - T_2}{2} \right) \right] + C_3 \left(\frac{T_3 - T_2}{2} \right)}{T_3}$$

The study author used the terms Threshold Level of Lethal Effects (TLLE), Threshold Level of Observed Effect (TLOE), and No Observed Effect Concentration (NOEC), in compliance with OECD guidance requirements. In essence, the TLLE and TLOE are LOAEC values for lethal and non-lethal endpoints, respectively. However, in this DER, the results in the Executive Summary and Conclusions sections were reported terms of NOAEC and LOAEC, as used by EPA in ecotoxicity studies.

It was reported in the statistical methods section of the study report that one of the endpoints assessed was sub-lethal effects at 28 days; however, it was also reported that as no sub-lethal effects were observed at 28 days, then the TLOE (for sub-lethal effects) was calculated using survival data. The visually-determined LOAEC for sub-lethal effects (based on clinical signs of toxicity observed at the 2.52 and 5.59 mg ai/L levels) is coincidentally equivalent to the TLOE reported in this study.

Test samples (aqueous) were cleaned-up using a pre-conditioned Bond-Elut cartridge. The cartridge was allowed to dry, rinsed with hexane (discarded), and then eluted with ethyl acetate. The resultant extract was blown to dryness under nitrogen, and re-dissolved with ethyl acetate. Samples not analyzed immediately were refrigerated (for an unspecified period of time) prior to analysis of the *cis* isomer using gas chromatography equipped with nitrogen/phosphorus detection (GC/NPD). The concentration of total metconazole was mathematically determined by the following formula: mg ai/L = mg *cis* isomer / 0.833 x 0.979, where 0.833 is the percentage of *cis*-isomer in the test substance and 0.979 is the percentage of total *cis* and *trans*-isomers in the test substance.

No insoluble material was noted in any test vessel during the definitive study. Insoluble material was observed attached to the liquid level control probes in the diluter toxicant mixing vessel throughout the test. The calculated concentration of the primary stock solution averaged 104% of the nominal concentration, and the calculated concentration of the secondary stock solution averaged 90% of the nominal concentration.

In-life dates for the range-finding study were March 11-18, 1996, and for the definitive study were April 30 – May 28, 1996.

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G. CONCLUSIONS:

This study is scientifically sound, but does not fulfill any current U.S. EPA guideline requirements and therefore will be treated as SUPPLEMENTAL. The 28-day LC_{50} was 1.71(1.16-2.53) mg ai/L. Based on clinical signs of toxicity and mortality at the ≥ 2.53 mg ai/L treatment levels, the NOAEC was 1.16 mg ai/L. No treatment-related effects on terminal growth of surviving fish (from the 0.511 and 1.16 mg ai/L levels) were observed.

28-day LC_{50} : 1.71 mg ai/L

95% C.I.: 1.16-2.53 mg ai/L

Probit slope: N/A

95% C.I.: N/A

NOAEC: 1.16 mg ai/L

LOAEC: 2.53 mg ai/L

Endpoint(s) affected: mortality and clinical signs of toxicity

III. REFERENCES:

Boeri, R.L. *et al.* 1996. Validation of Method FAMS 058-01 for the Determination of CL 900,768 Applied as Technical Material and in a 60 g/L SL Formulation (CARAMBATM fungicide) in Water and Aquatic Plant Growth Media. American Cyanamid Study Number 954-95-210.

EEC. 1992. Official Journal of the European Communities, Part C: Methods for the determination of Ecotoxicity. EEC Method C.1., Prolonged fish toxicity test.

Gulley, D.D., A.M. Boelter, and H.L. Bergman. 1990. TOXSTAT Version 3.3. Fish Physiology and Toxicology Laboratory, University of Wyoming, Laramie, Wyoming.

OECD. 1981. Decision of the Council Concerning the Mutual Acceptance of Data in the Assessment of Chemicals. Annex 2. OECD Principles of Good Laboratory Practice. C(81)30 (Final).

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

	EXPOSED	DEAD	DEAD	PROB. (PERCENT)
8.979999	20	20	100	9.536742E-05
5.59	20	20	100	9.536742E-05
2.53	20	20	100	9.536742E-05
1.16	20	0	0	9.536742E-05
.511	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.16 AND 2.53 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.713126

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

length of surviving fish

File: 84241 Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	51.7550	CALCULATED t VALUE =	1.7156
GRP2 (BLANK CRTL) MEAN =	50.1250	DEGREES OF FREEDOM =	38
DIFFERENCE IN MEANS =	1.6300		

TABLE t VALUE (0.05 (2),40) = 2.021 NO significant difference at alpha=0.05

TABLE t VALUE (0.01 (2),40) = 2.704 NO significant difference at alpha=0.01

length of surviving fish

File: 84241 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	2	6.484	3.242	0.392
Within (Error)	57	470.833	8.260	
Total	59	477.317		

Critical F value = 3.23 (0.05,2,40)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

Data Evaluation Report on the Toxicity of Metconazole Technical (Isomer Mix) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number {.....}

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length of surviving fish

File: 84241 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	51.755	51.755		
2	0.511	51.890	51.890	-0.149	
3	1.16	51.135	51.135	0.682	

Dunnett table value = 1.97 (1 Tailed Value, P=0.05, df=40,2)

length of surviving fish

File: 84241 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	20			
2	0.511	20	1.790	3.5	-0.135
3	1.16	20	1.790	3.5	0.620

length of surviving fish

File: 84241 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2					
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	20	51.755	51.755	51.823
2	0.511	20	51.890	51.890	51.823
3	1.16	20	51.135	51.135	51.135

length of surviving fish

File: 84241 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2					
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	51.823				
0.511	51.823	0.074		1.68	k= 1, v=57
1.16	51.135	0.682		1.76	k= 2, v=57

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s = 2.874

Note: df used for table values are approximate when $v > 20$.

wet weight of surviving fish

File: 8424w Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	3	16	18	19	4

Calculated Chi-Square goodness of fit test statistic = 2.8481

Table Chi-Square value ($\alpha = 0.01$) = 13.277

Data PASS normality test. Continue analysis.

wet weight of surviving fish

File: 8424w Transform: NO TRANSFORMATION

Shapiro-Wilks test for normality

***** Shapiro-Wilks Test is aborted *****

This test can not be performed because total number of replicates is greater than 50.

Total number of replicates = 60

wet weight of surviving fish

File: 8424w Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 1.49

Closest, conservative, Table H statistic = 4.9 ($\alpha = 0.01$)

Used for Table H ==> R (# groups) = 3, df (# reps-1) = 15

Actual values ==> R (# groups) = 3, df (# avg reps-1) = 19.00

Data PASS homogeneity test. Continue analysis.

Data Evaluation Report on the Toxicity of Metconazole Technical (Isomer Mix) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

wet weight of surviving fish

File: 8424w Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 0.76
Table Chi-square value = 9.21 (alpha = 0.01)
Table Chi-square value = 5.99 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 19.00
Used for Chi-square table value ==> df (#groups-1) = 2

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

wet weight of surviving fish

File: 8424w Transform: NO TRANSFORMATION

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN = 2.2940 CALCULATED t VALUE = 1.1458
GRP2 (BLANK CTRL) MEAN = 2.1225 DEGREES OF FREEDOM = 38
DIFFERENCE IN MEANS = 0.1715

-
TABLE t VALUE (0.05 (2),40) = 2.021 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),40) = 2.704 NO significant difference at alpha=0.01

wet weight of surviving fish

File: 8424w Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE DF SS MS F

Between 2 0.431 0.215 1.243
Within (Error) 57 9.889 0.173

Total 59 10.319

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Critical F value = 3.23 (0.05,2,40)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 :All groups equal

wet weight of surviving fish

File: 8424w Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	2.294	2.294		
2	0.511	2.187	2.187	0.814	
3	1.16	2.086	2.086	1.578	

Dunnett table value = 1.97 (1 Tailed Value, $P=0.05$, $df=40,2$)

wet weight of surviving fish

File: 8424w Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	20			
2	0.511	20	0.259	11.3	0.107
3	1.16	20	0.259	11.3	0.208

wet weight of surviving fish

File: 8424w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2					
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	20	2.294	2.294	2.294
2	0.511	20	2.187	2.187	2.187
3	1.16	20	2.086	2.086	2.086

wet weight of surviving fish

File: 8424w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2					
	ISOTONIZED	CALC.	SIG	TABLE	DEGREES OF

Data Evaluation Report on the Toxicity of Metconazole Technical (Isomer Mix) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number {.....}

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IDENTIFICATION	MEAN	WILLIAMS	P=.05	WILLIAMS	FREEDOM
neg control	2.294				
0.511	2.187	0.812		1.68	k= 1, v=57
1.16	2.086	1.575		1.76	k= 2, v=57

s = 0.417

Note: df used for table values are approximate when v > 20.

Data Evaluation Report on the Toxicity of Metconazole Technical (Isomer Mix) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number {.....}

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APPENDIX II: RESULTS OF TWA CALCULATIONS:

Nominal Concentration (mg ai/L)	Time (Day)	Measured Replicate 1 (mg ai/L)*	Measured Replicate 2 (mg ai/L)*	Mean Measured Concentration (mg ai/L)	TWA (mg ai/L)
0.64	0	0.583	0.623	0.603	0.511
	7	0.510	0.531	0.521	
	14	0.518	0.532	0.525	
	21	0.410	0.439	0.425	
	28	0.545	0.550	0.548	
1.2	0	1.26	1.29	1.28	1.16
	7	1.06	1.12	1.09	
	14	1.26	1.19	1.23	
	21	1.16	1.14	1.15	
	28	1.02	1.09	1.06	
2.5	0	2.74	2.66	2.70	2.53
	7	2.66	2.35	2.51	
	14	2.40	N/A	2.40	
5.1	0	5.50	5.88	5.69	5.59
	7	5.48	5.50	5.49	
10	0	9.26	9.78	9.52	8.98
	7	8.63	8.24	8.44	

*When more than one result (i.e., re-analysis) was provided, the mean of all analyses was used.